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# MITOCHONDRIAL DNA VARIATION OF THE SUMATRAN ELEPHANT POPULATIONS IN SUMATERA, INDONESIA

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## ABSTRACT

Genetic analysis of Mitochondrial DNA diversity in Sumatran elephant (*Elephas maximus sumatranus*) was conducted. A 630 bp segment of mitochondrial DNA was amplified from 105 different Sumatran elephant samples from 5 locations in Sumatera (Bentayan, Sugihan, Bukit Salero Lahat, Seblat, Way Kambas) using a set of primers: MDL3 (5'-CCCACAAT-TAATGGGCCC-GGAGCG-3') and MDL5 (5'-TTACATGAATTGGCAGCCA-ACCAG-3'). The objectives of this study were to generate mitochondrial DNA D-loop sequences for all available Sumatran elephant samples and to define haplotypes and nucleotide sequence diversity of the different Sumatran elephant populations. The nucleotide sequence of a total of 105 PCR products were successfully determined with an average length of 616 bp. However, mitochondrial DNA fragments for this analysis used the first 601 bases. Six different haplotypes (BP, BT, BS, BR, BX and BY) were identified in Sumateran elephant populations. The majority of the sampled individuals carried haplotype BT. BX and BY are most likely novel derived haplotypes. All haplotypes, except for the haplotype BP belong to the Sumatera clade. The haplotype BX was derived from the haplotype BT, and the haplotype BY was derived from the haplotype BS by one transversion, respectively. All the other substitutions identified in this network were transitions. The haplotype BP is widely distributed from Sri Lanka, Sumatera, Peninsular Malay and China. Although reported to be distributed in Sumatera and Peninsular Malay the haplotype BU was not detected among the samples analysed in this study. Genetic distances within populations in Bentayan, Bukit Salero Lahat, Seblat, Sugihan and Way Kambas ranged from 0.0000 - 0003, and the genetic distance between the populations that is 0.0000 - 0.0022. The distance between haplotypes of different Sumatran elephant populations was shown to be low. The diversity of haplotypes and nucleotides in Sumatera Island were low, the highest diversity was found in elephants sampled in the region of Bukit Salero Lahat and the lowest was found in elephants from Bentayan and Sugihan. Overall, the results of analysis of Fu and Li's F*test statistic indicates that the population of Sumatran elephants in Sumatra is -0.78871, which suggests that there is no inbreeding. However, the results are not significant (P> 0:10) and additional studies are required to confirm this finding.

**Key words:** Sumatran elephant, *Elephas maximus sumatranus*, mitochondrial DNA, haplotype

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## INTRODUCTION

The Asian elephant is an endangered animal and listed on Appendix I of CITES (Convention on International Trade in Endangered Species) of Wild Fauna and Flora. Sumatran elephants (*Elephas maximus sumatranus*) is one of four different Asian elephant subspecies in Indonesia. Today, the Sumatran elephant is found in seven main provinces, namely Nanggroe Aceh Darussalam, North Sumatra, Riau, Jambi, Bengkulu, South Sumatra, and Lampung (Soehartono *et al.* 2007). The main serious threats to Sumatra's elephant populations are among others forest/habitat loss, illegal hunting, poaching for ivory, and habitat degradation due to conversion of critical forest habitat to agriculture/plantation areas. Also many elephant populations are trapped in small pockets which are not enough to support their life, and the condition has sparked conflict between humans and elephants. Elephants raid people's crop, destroy their houses, and sometimes kill or injure people. In response, elephants are killed and the local communities persuade the government to catch and remove elephants from the wild. Therefore, information on status and distribution of Sumatran elephant populations, including information on genetic diversity of the remaining populations are urgently needed to determine conservation policy.

Molecular technology development at the moment has increased the efficiency and accuracy in the genetic characterization study among breeds of animals. Mitochondrial DNA (mtDNA) sequences have been extensively and successfully used to determine genetic diversity. Mitochondrial DNA is passed to offspring by matrilineal inheritance of mitochondria via the oocyte. Different regions of the mtDNA evolve at different rates. The area Displacement-loop (D-loop) has variation which is quite high, more polimorphic compared to other area mtDNA (Ishida *et al.* 1994; Quinn & Wilson 1993). As statement revealed by Brown *et al.* (1982), Quinn dan Wilson (1993) and Akhisinomiya *et al.* (1994), D-loop area is often used for phylogenetic analysis, both inside the species and among species. Therefore, much genetic variation can be expected between individuals of the same species.

Molecular techniques have been used to evaluate the diversity of Asian elephants across or within populations. Maternal inherited mitochondrial DNA (mtDNA) (Fernando & Lande 2000; Fernando *et al.* 2000, 2003; Fleischer *et al.* 2001; Vandebona *et al.* 2002; Vidya & Sukumar 2005; Vidya *et al.* 2005a, b, 2007; Fickel *et al.* 2007) revealed several haplotypes, which belong to two haplogroups or clades. In Thailand, eight haplotypes from two clades were found in 82 captive elephants (Lertwatcharasarakul *et al.* 2003), while mtDNA control region sequences from 78 captive elephants represented 20 haplotypes (Fickel *et al.* 2007). Fernando *et al.* (2000, 2003) identified 27 different haplotypes within the Asian elephant population by sampling over three hundred elephants from Sri Lanka, Bhutan/North India and Laos/Vietnam, Malaysia, Thailand, Bangladesh and Cambodia. These haplotypes are/clustered into two well-differentiated assemblages/clades, α and β. These assemblages corresponded to the clades that were found by Vidya *et al.* (2005) using mtDNA sequencing. Fleischer *et al.* (2001) also found these two distinct assemblages by sequencing mtDNA.

However, studies that analyze the Sumatran elephant based on mitochondrial DNA are still limited, such as the studies of Fernando *et al.* (2003) and Fleischer *et al.* (2001). There was also a study among 27 elephants (17 samples from Alas Nepal and 10 samples from Way Kambas) reported by Okayama *et al.* (2001) on mitochondrial DNA analysis of Sumatran elephant, stated that Sumatran haplotypes were more similar to Sri Lanka haplotypes than mainland Asian haplotypes. Haplotype frequencies were different between populations of Alas Nepal and Way Kambas. Unfortunately, there were no results on genetic differentiation of Sumatran population, because sampling location and size are too small to estimate genetic differentiation in Sumatra.

Furthermore, genetic analysis of the Sumatran elephant populations comprehensively sampled from Aceh to Lampung has not yet been reported. Therefore, a research on Mitochondrial DNA analysis of genetic diversity within maternal lines of different populations of Sumatran elephant (*Elephas maximus sumatranus*) was conducted in this study, in order (1) to generate mtDNA D-loop sequences for all the Sumatran elephant samples under this study and (2) to provide information on haplotypes and degree of nucleotide sequence diversity of Sumatran elephant populations. To conduct the study, the highly variable D-loop region of mtDNA on 105 samples of Sumatran elephant from 5 locations in Sumatra was analyzed in this study. All results obtained in this study should be useful for conservation strategies, particularly for Sumatran elephants. It is believed that diversity is only secure when diverse conservation strategies are employed.

## MATERIALS AND METHODS

### Samples and study areas

A total of one hundred and five (105) DNA material blood of Sumatran elephants (*Elephas maximus sumatranus*) were collected in 5 different locations of Elephant Conservation Center (Table 1). The samples were preserved in 96 % absolute ethanol and stored at the Bank of Material DNA for Indonesian Fauna, Genetic Laboratory, Division of Zoology, Research Center for Biology-LIPI. The samples were collected in 2000 by T. Okayama *et al.* in collaboration between Biodiversity Conservation Project (BCP) - Japan International Cooperation Agency (JICA) and Research Center for Biology (RCB) - LIPI. A list of the samples is shown in Table 1.

Table 1. List of Samples of Sumatran elephants (*Elephas maximus sumatranus*) collected from 5 locations.

| No. | Elephant Conservation Center | Province | Total Samples |
|---|---|---|---|
| 1 | Sugihan (SU) | South Sumatra Province | 18 |
| 2 | Bentayan (BE) | South Sumatra Province | 20 |
| 3 | Seblat (SE) | Bengkulu Province | 21 |
| 4 | Bukit Serelo Lahat (BSL) | South Sumatra Province | 24 |
| 5 | Way Kambas (WK) | Lampung Province | 22 |
| TOTAL SAMPLES | | | 105 |

**DNA extraction, amplification and sequencing**

DNA was extracted from whole blood using Qiagen "DNeasy®Blood & Tissue Kit. Amplification of mitochondrial DNA was conducted by the method of Polymerase Chain Reaction (PCR), using Thermal Cycler 2700 (Applied Biosystems). A 630 bp segment of mtDNA was amplified using a set of primers, MDL3 (5'-CCCA-CAATTAAT-GGGCCCGGAGCG-3') and MDL5 (5'-TTACATGAATTGGCAG-CCAACCAG-3') (Fernando *et al*., 2000), and subsequent nucleotide sequencing of the amplified fragment. These primers amplify a 630-bp fragment of mtDNA, including the D-loop. Fernando *et al.* (2000 & 2003) stated that the first 109 bp of the fragment code is for the C terminal end of cytochrome b, the next 135 bp code for threonine and proline tRNA's, the rest of the fragment corresponds to the non-coding control region that is the D-loop. PCR amplification was performed using a mixture of 30 µl reactions containing of 50 mg sample DNA, PCR buffer 1 x 200 UM dNTPs, 2 mM $MgCl_2$ and 1 unit Taq DNA polymerase (Fermentas, Native with BSA).

The amplified fragment was purified, and subsequently the nucleotide sequence was determined. The nucleotide sequences obtained, were aligned, edited and analyzed including definition of genetic diversity, genetic distance (estimate of the number of nucleotide substitutions per nucleotide site between two sequences), and phylogenetic analysis by the neighbour joining method. The haplotypes determined in this study was compared to the haplotypes identified by both Fernando *et al.* (2000 & 2003). and Vidya *et al.* (2005). The accession numbers for the Fernando *et al.* haplotypes as deposited in GenBank (http://www.ncbi.nlm.nih.gov) are: AY245538 and AY245802 to AY245827, and for Vidya *et al.* haplotypes as deposited in GenBank are AY365432 and AY365433. Trees were rooted using wooly mammoth (GeneBank NC007596, Krause *et al.* 2006 and DQ316067, Rogaev *et al.* 2006), African forest elephant (GeneBank JF827275, Ishida *el al*., 2011) and African savanna elephant GeneBank AF527654, Eggert *et al.* 2002 , and GeneBank DQ316069, Rogaev *et al.* 2006) as an outgroup.

**Data analysis**

A fragment of the D-loop mtDNA corresponding to the first 601 bp was used for analysis in this study. Nucleotide sequence data was obtained after editing of the fragment D-loop sequence. Sequence data was analysed with various kinds of computer software.

Chromas which available on http://www.technelysium.com.au/chromas.html was used for *viewing* and editing the sequence result. The ClustalX 1.83 was used for multiple alignment of sequences (Thompson *et.al.* 1997; and obtained from ftp://ftp-igbmc:u-strasbg.fr/pub/ClustalX). MacClade 4.0 was used to make the polymorphic sites (Maddison and Maddison, 2000 and available on http://ag.arizona.edu/macclade/macclade.html). Molecular Evolutionary Genetic Analysis (MEGA) version 3.0 was used for phylogenetic and analysis of the molecular evolution (Kumar *et.al.* 2004, available on http://www.megasoftware.net/), while Network analysis was used for illustrating haplotype diversity i.e. NETWORK 4.1.0.8 (Bandelt *et.al.* 1999).

Genetic diversity indices of Sumatran elephants including haplotype diversity, haplotype frequencies, nucleotide diversity, and FU and LI's F*test (1993), were carried out using DnaSP version 4.0 (Rozas *et al*., 2003; available at http://www.ub.es/dnasp).

## RESULTS AND DISCUSSIONS

A total of 105 PCR products from the samples of Sumatran elephants were successfully sequenced, with an average length of about 616 base pairs. The mtDNA fragments used for this analysis corresponds to the first 601 bases. Table 2 and Figure 1 show the six (6) haplotypes found in Sumatran elephants (BP, BT, BS, BR, BX and BY) and identified at 23 the sites of the polymorphic (variable sites) (Table 3). All haplotypes except for the haplotype BP (BSL-20 and BSL-23) are belonging to the Sumatera clade. The haplotype BP which belongs to the continental clade (Fig. 2) is widely distributed from Sri Lanka, Sumatera, Peninsular Malay (Fernando *et al*. 2000, 2003, Vidya *et al*. 2009), and China (Yonezawa *et al*. unpublished). Although Fernando *et al*. (2000 & 2003), and Vidya *et al*. (2009) reported that the haplotype BU is distributed in Sumatera and Peninsular Malay, but BU haplotype could not be detected in the samples analysed in this study.

Table 2. Haplotype distribution of 105 sequence of mitochondrial DNA

| No | Haplotype | Individual with same haplotypes | Number haplotypes | % |
|---|---|---|---|---|
| 1 | BT | BE8,BE19,BE17,BE15,BE5,BE7,BE2,BE4, BE10,BE1,BE13,BE6,BE16,BE14,BE20, BE18, BE3,BE9,BE11,BE12 | 20 | |
| | | BSL17,BSL8,BSL12,BSL1,BSL7,BSL16, BSL13,BSL2,BSL4, BSL11,BSL15,BSL19,BSL14, BSL10,BSL6, BSL9, BSL3, BSL5, BSL18 | 19 | 91,40% |
| | | SU2, SU5,SU11, SU6,SU15,SU3,SU10,SU17,SU8,SU12,SU13,SU7,SU9,SU1,SU4,SU14, SU16, SU18 | 18 | |
| | | WK19,WK16,WK1,WK20,WK21,WK18,WK7,WK14,WK15, WK9,WK12, WK17, WK5, WK11, WK3, WK8, WK13 | 19 | |
| | | SE9,SE20,SE12,SE4,SE14,SE18,SE2,SE13, SE5,SE3,SE17,SE15,SE21,SE8, SE7,SE6,SE10,SE16,SE1,SE11 | 20 | |
| 2 | BS | WK10,WK22 | 2 | 1,90% |
| 3 | BR | BSL21,BSL22,BSL24 | 3 | 2,90% |
| 4 | BP | BSL20,BSL23 | 2 | 1,90% |
| 5 | BX | SE19 | 1 | 0,95% |
| 6 | BY | WK4 | 1 | 0,95% |
| | | TOTAL | 105 | 100% |

NOTES: WK = Way Kambas, SU = Sugihan, SE = Seblat, BSL= Bukit Selero Lahat,BE = Bentayan

The genetic diversity (haplotype diversity, nucleotide diversity and haplotype frequencies) were analyzed for each region (BE, BSL, SE,SU, and K), and whole Sumatera Island (Table 3). These analyses were carried out by using DnaSP version 4.10 (Rozas *et al.* 2003). Based on the whole data sequence, the nucleotide diversity in Sumatra is 0.00092, in Way Kambas (WK) 0.00056, in Seblat (SE) 0.00016, the Bukit Salero Lahat (BSL) 0.00309, and zero (0) in Bentayan (BE ) and Sugihan (SU). Haplotype diversity on the island of Sumatra is 0.164, Way Kambas (WK) is 0.255, Seblat (SE) is 0.095, the Bukit Salero Lahat (BSL) 0.366, and zero (0) in Bentayan (BE) and Sugihan (SU). The highest haplotype and nucleotide sequence diversity was found in elephants from the Bukit Salero Lahat (BSL) region, and the lowest is zero (identity) which was found in elephants from the Bentayan (BE) and Sugihan (SU) regions. This means, there is a lack of mtDNA diversity in elephants sampled from this region. There is only one type of haplotype, and consequently the haplotype frequency is 100%.

The conditions in the region of Sugihan and Bentayan might indicate inbreeding. Unfortunately, the analysis of Fu and Li's F * test cannot be performed in the regions of Sugihan and Bentayan, because genetic diversity = 0. Overall, the results of analysis of Fu and Li's F * test statistic indicates that the population of Sumatran elephants is -.78871, which means there is no inbreeding, but not significant at P> 0:10. The result of genetic distance calculations, showed that genetic distances within regions/ populations in Bentayan, Bukit Salero Lahat, Seblat, Sugihan and Way Kambas ranged from 0.0000 - 0003, is the genetic distance between the populations that is 0.0000 - 0.0022. Thus, both genetic distance within populations and among the different populations of Sumatran elephants are very low. The distance is also counted among the eight haplotypes, consisting of 6 haplotypes (BP, BT, BS, BR, BX, BY) which are found in the Sumatran elephant, BD (Borneo haplotype) and BU haplotypes reported by Fernando *et al.* (2000 & 2003), and Vidya *et al.* (2009*)* in Sumatra. The highest haplotype distance shows 0.17 and the lowest shows 0.0002. A limitation with this type of analysis concerns the maternal inheritance of mitochondria as well as the population structure of elephants with dominant females. Asian elephants live in matriarchal societies consisting of family groups of several related females and their (juvenile) offspring. Males leave these groups when they reach puberty (Fernando & Lande 2000; Vidya & Sukumar 2005). Hence, although less commonly used, Y-chromosome characters are required in its context as male lineage (paternal lineages) just like mitochondrial DNA in female lineage (maternal lineages). Therefore, additional study based on Y chromosome may be needed to obtain a more comprehensive results in this study.

Figure 2 shows the median joining network (Bandelt *et al.* 1999) by using the NETWORK version 4.5.1.6 (http://www.fluxus-engineering.com). Most of the sampled individuals are the haplotype BT. The haplotype BX and BY are novel haplotypes. From Figure 1, the haplotype BX was derived from the haplotype BT, and the haplotype BY was derived from the haplotype BS by one transversion, respectively. The other substitutions in this network were transitions.

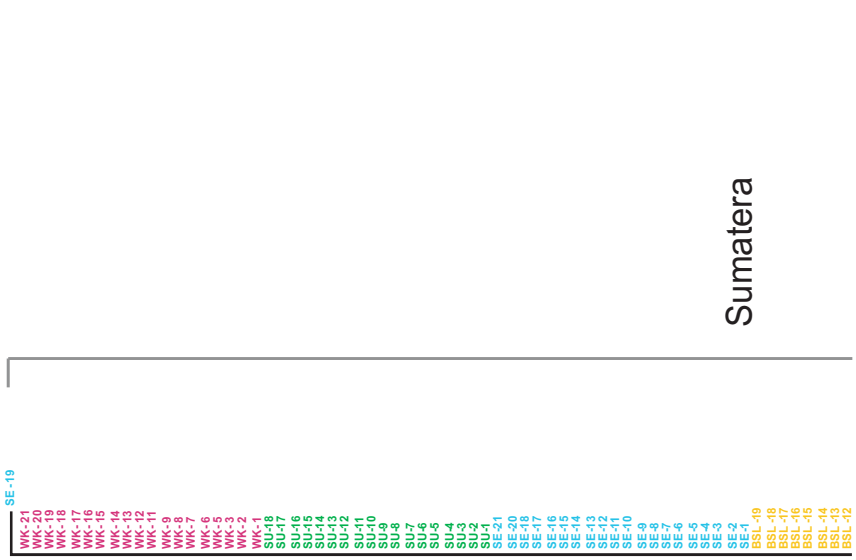


Figure 1. Neighbour Joining Tree (NJ tree) of Sumatran elephants and other Asian elephants from Malay, Continental Asia and Borneo.

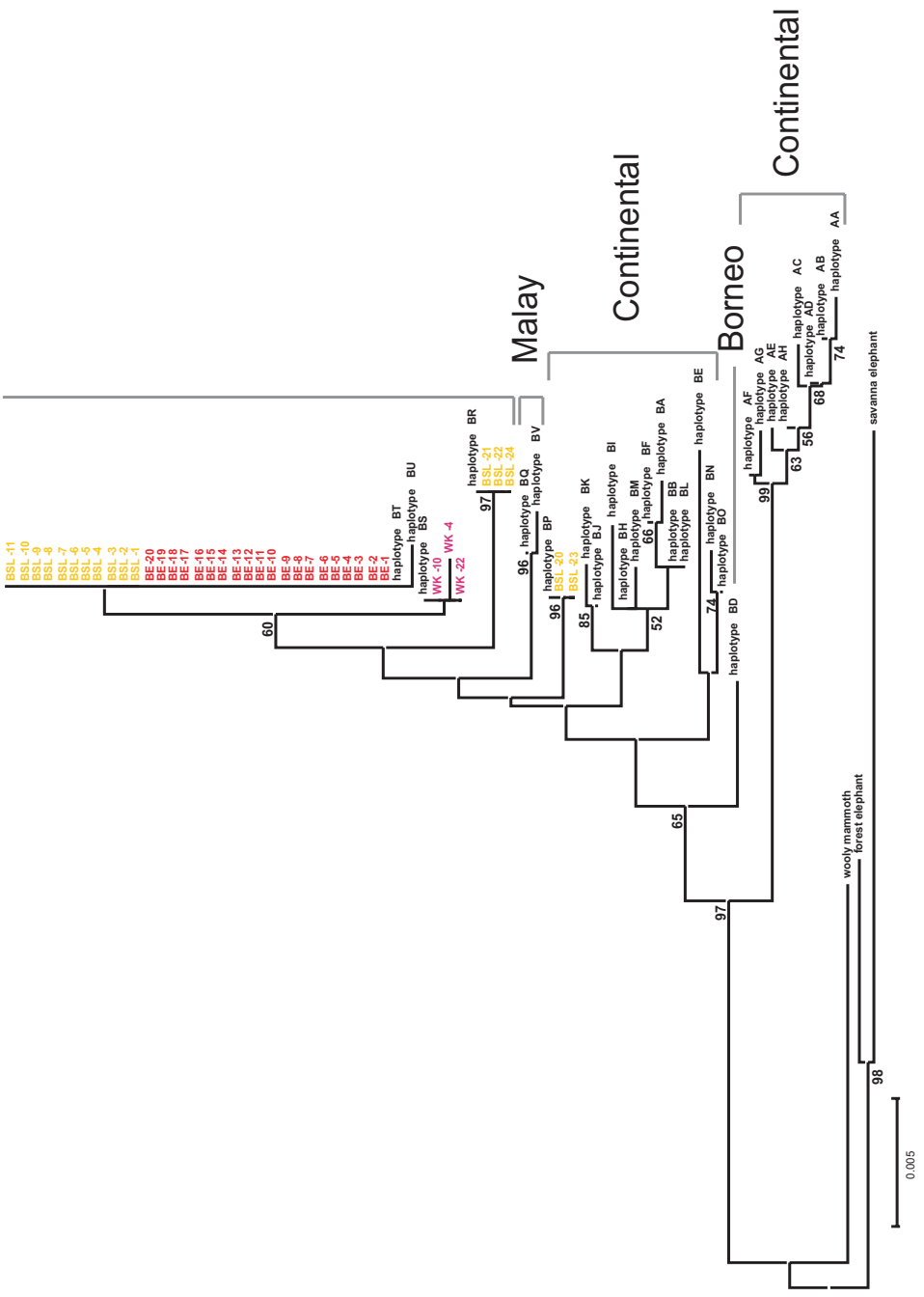
Malay
Continental
Borneo
Continental
BSL -11
BSL -10
BSL -9
BSL -8
BSL -7
BSL -6
BSL -5
BSL -4
BSL -3
BSL -2
BSL -1
BE-20
BE-19
BE-18
BE-17
BE-16
BE-15
BE-14
BE-13
BE-12
BE-11
BE-10
BE-9
BE-8
BE-7
BE-6
BE-5
BE-4
BE-3
BE-2
BE-1
haplotype BT
haplotype BS
haplotype BU
WK -10
WK -4
WK -22
haplotype BR
BSL -21
BSL -22
BSL -24
haplotype BQ
haplotype BV
haplotype BP
BSL -20
BSL -23
haplotype BJ
haplotype BK
haplotype BH
haplotype BI
haplotype BG
haplotype BM
haplotype BF
haplotype BA
haplotype BB
haplotype BL
haplotype BE
haplotype BO
haplotype BN
haplotype BD
haplotype AF
haplotype AG
haplotype AE
haplotype AH
haplotype AC
haplotype AD
haplotype AB
haplotype AA
wooly mammoth
forest elephant
savanna elephant
0.005

Table 3. Genetic Diversity indices of Sumatran elephants

| Region* | Hd (Haplotype diversity) | (Nucleotide diversity) | Number Haplotipe | Haplotype ** |
|---|---|---|---|---|
| Way Kambas/WK (22) | 0.255±0.116 | 0.00056±0.00028 | 3 | BS(0.09), BT(0.864), **BY (0.045)** |
| Sugihan/SU (18) | 0 | **0** | 1 | BT(1.00) |
| Seblat/SE (21) | 0.095±0.084 | 0.00016±0.00014 | 2 | BT(0.952), **BX (0.048)** |
| Bukit Salero Lahat/BSL (24) | 0.366±0.115 | 0.00309±0.00098 | 3 | BP(0.792), BR(0.125), BT(0.08 |
| Bentayan/BE (20) | **0** | **0** | 1 | BT(1.00) |
| Sumatera (105) | 0.164±0.049 | 0.00092±0.00032 | 6 | BP(0.019), BR (0.01), BS(0.019), BT(0.914), **BX(0.01), BY (0.01)** |

Note: Hd: Haplotype diversity (expressed as average ± 1SE)
Nd: Nucleotide diversity (expressed as average ± 1SE)
* The number of individuals sampled are shown within parentheses
** The frequencies of the haplotypes are shown within parentheses.
The bold characters indicate the novel haplotypes.

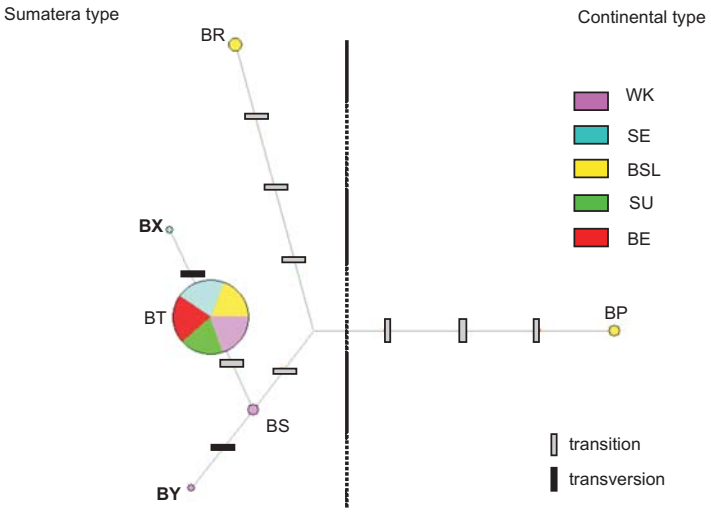


Figure 2. Median Joining Network

Note:
Sampling Locations:
WK = Way Kambas, SU = Sugihan , SE = Seblat, BSL = Bukit Selero Lahat, BE = Bentayan
Haplotypes: BR, BX, BT, BY

## CONCLUSIONS

Based on the analysis of mitochondrial DNA D-loop sequence from 105 samples of Sumatran elephants, it was concluded that Sumatran elephants within populations and among populations in Sumatra exhibit only limited or very low genetic diversity. This study provides a first definition of mtDNA diversity and is an important contribution. BP, BR, BS, and BT haplotypes, and 2 new haplotypes (BX and BY) are found in the Sumatran elephant. The distance between haplotypes of Sumatran elephant's population is also low. Moreover, additional samples may be needed to obtain significant conclusions. A more comprehensive conclusion may be obtained if also Y chromosome markers and autosomal markers are analyzed.

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## REFERENCES

Akishinonomiya F, Miyake T, Sumi S, Takada M, Ohno S, Kondo N. 1994. One subspecies of the Red Jungle Fowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. In: Proceedings National Academy of Sciences USA. 91: 12505-9.

Bandelt HJ, Forster P, Rohl A. 1999 Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16: 37-8.

Brown WM, Prager EM, Wang A, Wilson AC. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J Mol Evol 18:225-39.

Eggert LS, Rasner CA, Woodruff DS. 2002. The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. Proceedings of the Royal Society London, Biol Sci 269 (1504), 1993-2006.

Fernando P, Lande R. 2000. Molecular genetic and behavioural analysis of social organisation in the Asian elephant (*Elephas maximus*), *Behav Ecol Sociobiol* 48: 84-91.

Fernando P, Pfrender ME, Encalada SE, Lande R. 2000. Mitochondrial DNA variation, phyleogeography and population structure of the Asian elephant. *Heredity* 84: 362-72.

Fernando P, Vidya TNC, Payne J, Stuewe M, Davison G, Alfred RJ, Andau P, Bosi E, Kilbourn A, Melnick DJ. 2003. DNA analysis indicates that Asian elephants are native to Borneo and are therefore a high priority for conservation. *PloS Biology* 1 (1): 110-5.

Fickel J, Lieckfeldt D, Ratanakorn P, Pitra C. 2007. Distribution of haplotypes and microsatellite alleles among Asian elephants (*Elephas maximus*) in Thailand. Eur J Wildlife Res 53: 298-303.

Fleischer RC, Perry EA, Muralidharan K, Stevens EE, Wemmer CM. 2001. Phyleogeography of the Asian elephant based on mitochondrial DNA. *Evolution* 55 (9): 1882-92.

Ishida N. T Hasegawa, K. Takeda, M. Sagami, A. Onishi, S. Inumaru, M. Komatsu, H. Mukoyama. 1994. Polymorphic sequence in the D-loop region of equine mitochondrial DNA. Animal Genetics. 25:215-21.

Ishida Y, Oleksyk TK, Georgiadis NJ, David VA, Zhao K, Stephens RM, Kolokotronis SO, Roca AL. 2011. Reconciling apparent conflicts between mitochondrial and nuclear phylogenies in African elephants. PLoS ONE 6 (6), E20642.

Maddison DR, Maddison WP. 2000. MacClade 4: Analysis of phylogeny and character Evolution , 4.0. Sinauer Associates, Sunderland, MA.

Krause J, Paul HD, Joshua LP, Montgomery S, Helen, Ian B, Adrian ML, Ingo E, Svante P, Michael H .2006. Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. Nature 439, 724-7. (doi: 10.1038/nature04432).

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics. 5: 150-63. 2003. Haplotypes of Asian elephant (*Elephas maximus*) in Thailand based on cytochrome b gene. Kamphaengsaen.Acad J 1:33-39.

Okayama T, Sugardjito J, Yusuf I. 2001. Mitochondrial DNA analysis of Sumatran elephant. Progress Report Fiscal Year 200-2001. Biodiversity Conservation Project, JICA-LIPI.

Quinn TW, Wilson AC. 1993. Sequence evolution in and around the mitochondrial control region in birds. J Mol Evol 37:417-25.

Rogaev EI, Moliaka YK, Malyarchuk BA, Kondrashov FA, Derenko MV, Chumakov I, Grigorenko AP. 2006. Complete mitochondrial genome and phylogeny of Pleistocene mammoth Mammuthus primigenius. PLOS Biol 4, 403-10. (doi: 10.1371/journal.pbio.0040073)

Rozas J, Sánchez-DelBarrio JC, Messegyer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by coalescent and other methods. Bioinformatics 19: 2496-7.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-82.

Tonny S, Susilo HD, Gunaryadi D, Purastuti EM, Azmi W, Fadhli SC. 2007. Strategi dan Rencana Aksi Konservasi Gajah Sumatera dan Gajah Kalimantan 2007-2017. Departemen Kehutanan.

Vandebona H, Goonesekere NCW, Tiedemann R. 2002. Sequence variation at two mitochondrial genes in the Asian elephant (Elephas maximus) population of Sri Lanka. Mamm Biol 67:193-205.

Vidya TNC, Sukumar R. 2005. Social organization of the Asian elephant (*Elephas maximus*) in southern India inferred from microsatellite DNA. J Ethol 23:205-10.

Vidya TN, Fernando P, Melnick DJ, Sukumar R. (2005a). Population differentiation within and among Asian elephant (*Elephas maximus*) populations in southern India. Journal Heredity 94 (1), 71-80.

Vidya TNC, Fernando P, Melnick DJ, Sukumar R. 2005b. Population genetic structure and conservation of Asian elephants (*Elephas maximus)* across India. Anim Conserv 8: 377-88.

Vidya TNC, Varma S, Dang NX, Thanh TV, Sukumar R. 2007. Minimum population size, genetic diversity, and social structure of the Asian elephant in Cat Tien National Park and its adjoining areas, Vietnam, based on molecular genetic analyses. Conserv Genet 8:1471-78.

Vidya TNC, Sukumar R, Melniek DJ. 2009. Range-wide mtDNA phylogeography yields insights into the origins of Asian elephants. Proc R Soc B 276: 893-902. doi:10.1098/rspb.2008.1494, Published online 18 November 2008.